OBSERVATIONS UPON THE ENDAMEBAE OF THE MOUTH *

I. ENDAMOEBA GINGIVALIS (BUCCALIS)

PLATE 6

CHARLES F. CRAIG
Captain, Medical Corps, U. S. Army

From the Department Laboratory, Central Department, U. S. Army, Fort Leavenworth, Kan.

As a result of the statements of several recent writers and investigators that the endamebae so frequently encountered in the human mouth are pathogenic organisms and the cause of pyorrhea alveolaris and of many of the complications of so-called "oral-sepsis," interest has been reawakened in the study of these parasites, and several interesting and valuable contributions have appeared dealing with Endamoeba gingivalis (buccalis), a species commonly observed in the human mouth and held by some writers to be the cause of pyorrhea.

I have accepted the spelling Endamoeba instead of Entamoeba because of the fact that Joseph Leidy established the genus Endamoeba in 1879, to include parasitic amebae, the type species of the genus being Endamoeba blattae, of the cockroach. The genus Entamoeba was established in 1897, by Casagrandi and Barbagallo, who were evidently ignorant of Leidy's genus Endamoeba for parasitic amebae. By virtue of the law of priority the spelling of Leidy is to be preferred to that of Casagrandi and Barbagallo, and for this reason I adopt the name Endamoeba in place of Entamoeba.

During the past 9 months, through the kindness of Dental Surgeon Minot E. Scott, U. S. Army, stationed at this post, I have had the opportunity of studying the endamebae occurring in the mouth in patients suffering from pyorrhea alveolaris and other inflammatory conditions of the teeth and gums, and have also studied the endamebae occurring in normal mouths, and in this contribution I shall describe the morphology and life cycle, so far as I have been able to determine it, of Endamoeba gingivalis, Gros, which I believe to be identical with

* Received for publication September 22, 1915. Published with the permission of the Surgeon-General, U. S. Army.

Endamoeba buccalis, Prowazek. In a future contribution I hope to be able to describe the complete life cycle of what I believe to be a new species of endameba, occurring in this region, which I have provisionally named Endamoeba confusa. This species is characterized by its minute size, the fully developed vegetative organisms seldom measuring over 10 microns in diameter; the slight distinction between ectoplasm and endoplasm; a nucleus of the histolytica type but very minute; very sluggish motility under the most favorable conditions; and the absence of ingested bodies, which are so very frequently observed within Endamoeba gingivalis. This specific name "confusa" is given because of the liability of confusing this species with the smaller examples of Endamoeba gingivalis.

In all probability, further research will demonstrate that still other species of amebae occur in the human mouth, and it will be strange indeed if both Endamoeba histolytica and Endamoeba coli, the common intestinal endamebae of man, are not sometimes encountered in this locality, for it is through the mouth that infection of the intestine with these parasites occurs. It is also probable that some of the species of purely vegetative amebae will be found in the mouth, especially those belonging to the genus Vahlkampfia, for these have been found in the intestine of man, reaching that locality through the mouth. The probability of the occurrence in the mouth of these various species of amebae should not be lost sight of in the study of this subject and for this reason one should be very cautious in drawing conclusions regarding the species of amebae encountered in this region and their relation to diseases of the teeth, gums, and adjacent structures.

I. ENDAMOEBA GINGIVALIS GROS 1849. EM. V. PROWAZEK 1904

This, in all probability the most common species of endameba occurring in the mouth whether in health or disease, has recently attracted much attention because of its supposed relation to the etiology of pyorrhea alveolaris, and for this reason a detailed description of its morphology and life cycle is of interest to both physicians and dentists, especially as many of the descriptions in the literature are very brief and of little value to one who desires to differentiate this species from other species of endamebae.

HISTORY

Gros,[1] in 1849, described an ameba occurring frequently in the soft tartar of human teeth, characterized by blunt pseudopodia; a clear distinction between the ectoplasm and the endoplasm; the presence of ingested bodies, or globules,

[1] Bull. Soc. imp. de nat. de Moscow, 1849, 22, p. 549.

within the cytoplasm; and ameboid motility. The size of the organism, according to Gros, varied from 25 to 30 microns in diameter. He named the parasite Amoebea gengivalis.

In 1862, Steinberg[2] described an ameba which he found in the soft tartar and in the refuse material around the teeth, which, so far as his description indicates, was identical with the organism described by Gros, altho he evidently considered it a new species and named it Amiba buccalis. The same organism was again described as a new species by Grassi,[3] in 1879, who found it in material from the lesions of gingivitis. He named it Amoeba dentalis.

In 1904, v. Prowazek[4] gave a detailed description of an endameba occurring in the mouth, which he considered a new species, and which he named Entamoeba buccalis, evidently in ignorance of the prior use of this specific named by Steinberg in his description of the ameba found by him in the mouth, in 1862. Von Prowazek described the endameba observed by him as varying in size from 6 to 32 microns in diameter; actively motile; having a clear, distinct ectoplasm and an endoplasm containing many food vacuoles and much ingested material. The nucleus measured from 1.5 to 4.5 microns in diameter, was poor in chromatin, and had a well-defined, thick nuclear membrane and a deeply stained, very minute karyosome. Reproduction occurred by simple division, mitosis occurring first in the karyosome; but Prowazek also suggested that reproduction might occur by budding or gemmation, as he found in the endoplasm what he believed to be chromidia of nuclear origin. He was unable to find any reproduction cysts. He states that this parasite may be differentiated from Endamoeba coli by the clear hyaline ectoplasm and the differences in reproduction, and from Endamoeba histolytica by the very definite nuclear membrane and the greater rigidity of the nucleus.

Altho Prowazek gave a better description of this endameba than his predecessors, it is very evident that his Entamoeba buccalis is identical with the organism described previously by Gros, Steinberg, and Grassi, and it therefore follows that the specific name "buccalis" must be replaced by the name "gingivalis," first given the parasite by Gros.

In 1907, Lewald[5] stated that he had been able to demonstrate this endameba in the mouths of 75 of 100 individuals examined, and that they occurred in this region no matter how much care was given to the teeth.

But little attention was given Endamoeba gingivalis until the appearance of a paper by Barrett[6] upon the protozoa of the mouth in relation to pyorrhea alveolaris. In conjunction with Allen J. Smith he examined 46 patients suffering from suppurative conditions of the gums and the pericemental tissues and found Endamoeba gingivalis in all of them. He concludes the paper by stating his belief in the etiologic relation of this parasite to the lesions of pyorrhea and notes their disappearance from the lesions after the administration of emetin, a well-known amebacide.

Barrett's paper was followed by an important one from Chiavaro,[7] who studied this endameba under the direction of Grassi, in the latter's laboratory. He states that in all probability the organisms studied by Gros, Steinberg, Grassi, v. Prowazek, and himself are identical. Chiavaro is the first to describe

[2] Souremenaya meditsina., Kiev, 1862.
[3] Gazz. med. Ital.-Lomb., 1879, 39, p. 446.
[4] Arbeit. a. d. k. Gsndhts., 1904, 21, p. 42.
[5] Proc. New York Path. Soc., 1907. Cited, New York Med. Jour., 1915, 102, p. 281.
[6] Dent. Cosmos, 1914, 56, p. 948.
[7] Dent. Rev., 1914, 27, p. 1122.

encysted forms for Endamoeba gingivalis. He observed encystment in one case but gives no detailed description of the cysts, altho he pictures a cyst containing 2 nuclei. His drawings of the cystic forms are far from convincing, but it may be that he really observed the resistance cysts to be hereafter described.

In a later paper, Smith, Middleton, and Barrett[8] describe the occurrence of Endamoeba gingivalis in the tonsils in cases of chronic tonsillitis; and Bass and Johns,[9] as the result of their observations of this organism, concluded that it is the cause of pyorrhea, but added nothing to our knowledge of its morphology or life-cycle. In fact, the method of staining employed by them, as described in their paper, is valueless from a morphologic standpoint; with it one would be unable to differentiate this species from other species that occur in the mouth. From the description of the morphology of the parasite as given by them one is unable to be sure that they did not base their conclusions upon the observation of more than one species, while their unqualified statement that pyorrhea is due to Endamoeba gingivalis (buccalis) is entirely unjustified by any evidence that they were able to present in their contribution.

Several papers have very recently appeared in the dental journals dealing with the relation of this parasite to pyorrhea and other inflammatory conditions of the teeth and gums, the most important being those of Henrici and Hartzell,[10] Sarrazin,[11] and Price and LaRue,[12] and their conclusions will be discussed in that section of this contribution dealing with the relation of Endamoeba gingivalis to disease.

The most recent paper treating of Endamoeba gingivalis is that of Smith and Barrett,[13] in which they review the papers of the various investigators who have studied this parasite, and give a detailed description of its morphology and methods of reproduction. They agree that Endamoeba buccalis of v. Prowazek, is identical with the organism studied previously by Gros, Steinberg, and Grassi, and rightly state that by law of priority it should be called Endamoeba gingivalis instead of the commonly used name, Entameba buccalis. They state that they have observed resistance cysts, but no reproductive cysts. They believe that Endamoeba gingivalis is with great difficulty differentiated from Endamoeba histolytica and bring up the question of the possible identity of the two organisms.

GEOGRAPHICAL DISTRIBUTION

Endamoeba gingivalis apparently has a world-wide distribution, instances of infection having been reported wherever the parasite has been searched for. I, personally, have observed infections in individuals from many of the States of the Union and from the Philippines. It is more than probable that a careful examination of the natives of all countries would result in the demonstration of the presence of this parasite in the vast majority, and that such examinations would throw considerable light upon the question of the relation of this endameba to diseases of the teeth and gums.

[8] Jour. Am. Med. Assn., 1914, 63, p. 1746.
[9] Ibid., 1915, 64, p. 554.
[10] Jour. Nat. Dent. Assn., 1915, 2, p. 123.
[11] Items of Interest, 1915, 37, p. 404.
[12] Jour. Nat. Dent. Assn., 1915, 2, p. 143.
[13] Jour. Parasitol., 1915, 1, p. 159.

MORPHOLOGY

In considering the morphology of Endamoeba gingivalis it will be necessary to describe its appearance in the living condition and in stained preparations, and in order to differentiate the organism from other endamebae it is necessary to employ some method of wet fixation and staining, for in the living condition this species is differentiated only with extreme difficulty from certain stages in the life cycle of Endamoeba histolytica.

The life-history of Endamoeba gingivalis has not been completely worked out, but I have been able to determine that it passes through a vegetative stage of existence, during which it multiplies by simple division; a pre-cystic stage, in which I have not been able to detect any evidence of multiplication; and a cystic stage, in which, so far as I have been able to observe, no development occurs, the cysts at no time containing more than one nucleus. While it is too early to state definitely that reproduction cysts do not occur in this species of endameba, the only cysts that I have observed have been resistance cysts, evidently intended to protect the parasite under unfavorable conditions for development in the ordinary vegetative stage. This fact, of course, definitely differentiates this species from Endamoeba coli, in which cysts are produced containing from 8 to 16 nuclei, and from Endamoeba histolytica, which produces 4-nucleated cysts, and connects the species with the free-living amebae, in which, under normal conditions, only resistance cysts are formed.

MORPHOLOGY OF ENDAMOEBA GINGIVALIS IN THE LIVING CONDITION

The Vegetative Stage.—The vegetative forms of Endamoeba gingivalis in the living condition, vary in size from 7 to 35 microns in diameter, the average size being from 12 to 20 microns in diameter. Most authorities state that the size varies from 6 to 30 microns in diameter, and Smith and Barrett, in their latest paper, give the diameter as from 30 to 35 microns. I believe that most of the organisms in this stage of development that have been described as measuring less than 10 microns in diameter were not Endamoeba gingivalis but Endamoeba confusa, the new species I have already referred to, which seldom measures more than 10 microns in diameter, while the smaller individuals often measure from 5 to 6 microns in diameter. As regards the very large forms of Endamoeba gingivalis described, measuring from 40 to 60 microns in diameter, I am inclined to believe, with Smith and Barrett, that such forms are simply anomalous individuals; but the possibility of the occurrence of both Endamoeba coli and Endamoeba histolytica in the mouth should be remembered in differentiating these large forms of a species which normally is considerably smaller than either of the parasites named.

When resting, Endamoeba gingivalis is generally circular in shape, altho it may be irregular, but when in motion it is always irregular in outline. Oval forms are frequently observed, especially in stained preparations.

Motility, under favorable conditions, is marked in this species, but I am unable to agree with Smith and Barrett that motility is as marked as in Endamoeba histolytica. While, as a rule, motility is more pronounced in this species than in Endamoeba coli, I have never observed the very active motility commonly noted in Endamoeba histolytica. As a matter of fact, Endamoeba gingivalis is a rather sluggish species, the vast majority of the organisms simply sending out pseudopodia with little or no progressive motion. Examples, however, are observed which have a rather rapid progressive motion, in a definite direction; in such instances the pseudopodia are finger-shaped and longer than those usually observed.

The cytoplasm of Endamoeba gingivalis is divided into a clear outer portion, the ectoplasm, and a granular inner portion, the endoplasm. These two portions are plainly visible only when motility is present, the ectoplasm forming the pseudopodia. At such times there is a clear distinction between ectoplasm and endoplasm, but in this species the ectoplasm is much less refractive than it is in Endamoeba histolytica, approaching in appearance the ectoplasm of Endamoeba coli.

The pseudopodia, which are always formed by the ectoplasm, vary much in shape. Sometimes, when motility is most pronounced, the pseudopodia are long and slender, or finger-like, while at other times they are very short and blunt. Often endamebae are observed in which several pseudopodia are extruded at the same time, and when this occurs, the pseudopodia are always small and lobose in character and there is little or no progressive motion. In the vast majority of instances the pseudopodia are short and blunt, finger-shaped pseudopodia being observed only in organisms that are actively motile.

When the parasite is moving, the endoplasm flows into the ectoplasmic pseudopodia, the distinct division between these two portions, which exists as the pseudopodia are extruded, disappearing gradually, until, when motion ceases, there is no distinction between ectoplasm and endoplasm. Under high powers of the microscope, the ectoplasm is finely granular in appearance.

The endoplasm contains the nucleus, numerous food vacuoles, and ingested bodies of various nature. No contractile vacuole has been demonstrated by any observer.

In the living condition, in the vast majority of instances, the nucleus is invisible, this species resembling, in this respect, the histolytica stage of development of Endamoeba histolytica. Not infrequently an obscure body may be observed within the endoplasm which might be interpreted as the nucleus, but it is only very rarely that a definite nucleus can be demonstrated in the living organism. When the nucleus can be seen, it consists of a refractive ring of dots forming the nuclear membrane, within which may sometimes be seen one or more refractive granules.

The endoplasm contains numerous food vacuoles, which vary greatly in size in the same organism and generally contain granular material representing the remains of ingested bodies that have been partially digested. The smaller endamebae are less vacuolated than are the large organisms and the vacuoles are generally much smaller, while it is not rare to observe small endamebae of this species which are apparently free from vacuoles.

Endamoeba gingivalis is evidently very actively phagocytic, as shown by the fact that the vast majority of individuals contain numerous ingested bodies,

the most common of which are the nuclei of leukocytes, bacteria, and round or oval bodies, probably of protozoan nature. While this species has been described as actively phagocytic for red blood corpuscles, it has not been my experience that these cells are often present in the endoplasm, even tho the material containing the endamebae also contains blood. Altho there is no doubt that this species can phagocytose red blood corpuscles, it cannot be said that this occurs with anywhere near the frequency that it does in Endamoeba histolytica.

Small, round, and oval bodies are very frequently observed within the endoplasm which are obviously neither erythrocytes nor the nuclei of leukocytes, and some of the endamebae are literally packed with them. The exact nature of these bodies I have been unable to determine, but I believe that they represent some species of protozoan organism, and that they form a considerable part of the food supply of the parasite. Most authorities have apparently interpreted these bodies as the nuclei of leukocytes, but I see no reason for such interpretation, for there is certainly little resemblance between them and the nuclei of leukocytes when they are studied in stained preparations.

In the living endamebae I have never observed changes in the organism which I could be sure were reproductive in character. Rarely two organisms are observed side by side and this phenomenon might be interpreted as the result of division, but such an interpretation would only be a guess. However, I have observed a process which I regard as conjugation, in which two of the endamebae became united and there was a distinct interchange of cytoplasmic material, after which the organisms separated. This interchange of cytoplasmic substance was accompanied by very definite and marked currents within both endamebae, and ingested bodies within the organisms could be observed passing from one into the other.

I have also observed the process described by v. Prowazek and Lowenthal as reproduction by budding or gemmation, in which small particles of cytoplasm are apparently budded off from the periphery of the endameba. The process is exactly similar to that occurring in Endamoeba histolytica which for so long was interpreted as a method of reproduction, but which is now known to be degenerative in character. I am convinced that the same interpretation holds in the case of Endamoeba gingivalis—that organisms in which it is observed are not undergoing reproduction, the changes being due to degeneration of the parasites.

The Pre-Cystic Stage.—In this stage of development, in the living condition, the morphology of this species varies considerably from that observed in the vegetative stage. While I have chosen to call this a definite stage of development, it should be understood that, as yet, I have observed no evidences of multiplication in this stage, and that it is more than probable that the endamebae classified as belonging to this stage are simply organisms which are preparing to encyst.

In the living condition, the pre-cystic forms of Endamoeba gingivalis are markedly reduced in size, the average diameter seldom exceeding 10 to 12 microns. The cytoplasm is generally clear and free from vacuoles and ingested bodies, while a fairly definite nucleus is often observed, the nuclear membrane being represented by a circle of very refractive granules and the karyosome by a single refractive mass at or near the center of the nucleus. There is no differentiation of the cytoplasm into ectoplasm and endoplasm, and motility is practically absent. Rarely very small, blunt pseudopodia may be sluggishly extruded from the periphery of the organism, but when this occurs it will be noted that they are of the same appearance as the endoplasm. In the vast majority of instances

the pre-cystic forms are absolutely immotile, perfectly circular in shape, and composed of a clear, finely granular cytoplasm in which a nucleus, of the character just described, may or may not be visible.

The Cystic Stage.—The formation of resistance cysts occurs in this species, but I have never observed any evidence of reproduction within these cysts, so that I believe that cystic formation in Endamoeba gingivalis is a purely protective process and not a reproductive one, as in Endamoeba coli and Endamoeba histolytica.

In the living condition the cysts of Endamoeba gingivalis measure from 8 to 10 microns in diameter, as a rule, altho larger cysts are sometimes observed. They are perfectly circular in shape, and have a thin cyst wall, which may present a distinct double outline. The cytoplasm is clear and finely granular in appearance and there is no distinction into ectoplasm and endoplasm. A nucleus may or may not be distinguished within the cyst in the living condition, but in stained preparations a single nucleus is always present, except in those cysts that are degenerative in character.

Both pre-cystic and cystic forms of Endamoeba gingivalis occur very rarely, in my experience, and this fact may explain why cystic formation has only been noticed by one or two observers prior to this publication.

MORPHOLOGY OF ENDAMOEBA GINGIVALIS IN STAINED PREPARATIONS

While the study of living examples of Endamoeba gingivalis is of interest and value, the differentiation of this species from other endamebae, especially from Endamoeba coli and Endamoeba histolytica, is practically impossible, in many instances, unless stained preparations be studied. While one may make a diagnosis of the presence of endamebae by the examination of unstained material from the teeth and gums, it is practically impossible to be sure of the exact species present unless stained preparations are also studied, and to be of value in this direction these must be made by some method of wet fixation and hematoxylin staining. The method that I have found most useful and, at the same time, easiest of application, is fixation in Schaudinn's sublimate-alcohol solution and staining with the Rosenbusch-Hartmann stain for amebae. This method of staining was first brought to my attention by Major F. F. Russell, of the Medical Corps of the Army. It is much more convenient and simple than the other hematoxylin methods that I have employed heretofore. In fact, it is so easy of application and the results are so uniform that it may be employed by anyone at all familiar with laboratory technic, and it entirely obviates the necessity of resorting to such staining methods as Giemsa's or Wright's, preceded by dry fixation, which, because they have been considered less difficult of application, have been largely used in the past in the study of amebae. It is just as easy to employ the method of wet fixation and staining here recommended for amebae as the method advocated by Bass and Johns for the diagnosis of endamebae in the mouth, while this method has the very great advantage that, using it, one is able to differentiate the species of endamebae encountered, whereas with the method described by these writers it is impossible to do so.

The method is as follows:

1. Rapidly spread the material to be examined upon a glass microscopic slide or cover-glass, and before it has had time to dry, immerse the slide in Schaudinn's sublimate-alcohol fixing fluid, prepared as follows: Perchlorid of mercury is dissolved in boiling normal salt solution until a saturated solution

is obtained. Two parts of this solution are added to one part of absolute alcohol, the mixture is warmed, and the preparations are immersed in it for from 2 to 5 minutes.

2. Wash for a few minutes in 70% alcohol; 70% alcohol plus enough iodin to give it a port wine color; 70% alcohol; and distilled water.

3. Place preparations in a 3.5% solution of iron-alum in distilled water. In this they may remain over night, if necessary, but ½ hour is sufficient.

4. Wash thoroughly in distilled water.

5. Stain the preparations with the following lithium carbonate hematoxylin stain:

(*a*) A 1% solution of hematoxylin in 95% alcohol. This solution should not be used until 10 days after mixing.

(*b*) A saturated solution of lithium carbonate in distilled water. The two solutions are mixed for staining in the following proportion; of *a*, 10 c.c., and of *b*, 5 to 6 drops. The smears are stained with this mixture for from 5 to 20 minutes.

6. Wash thoroughly in distilled water.

7. Differentiate with a weak iron-alum solution. The solution given in Step 3 diluted with 3 parts of distilled water is recommended.

8. Wash thoroughly in distilled water; 95% alcohol; absolute alcohol; clear with xylol, and mount in neutral balsam.

At no time during the fixing and staining process should the preparations be allowed to dry or they will be valueless.

With the method described, Endamoeba gingivalis presents certain characteristics that distinguish it from other species of endamebae that I have studied. As the morphology of the parasite varies at different stages of development, it will be necessary to consider its appearance in the vegetative, pre-cystic, and cystic stages of development separately.

The Vegetative Stage of Development.—Stained specimens at this stage measure from 8 to 25 microns in diameter, altho larger forms may sometimes be encountered. The shape of the organism is usually round or oval, but may be irregular when fixation has occurred during the protrusion of a pseudopodium. There is no distinction between the ectoplasm and the endoplasm in the round or oval organisms, altho in the irregular forms the outer portion, or ectoplasm, forming the pseudopodium, stains a lighter gray than the endoplasm and appears very finely granular. With the staining method described the cytoplasm stains a brownish gray when the specimens are properly differentiated.

The nucleus, in stained preparations, is, in my opinion, wholly characteristic, and easily distinguished from the nucleus of either Endamoeba coli or Endamoeba histolytica, in both size and structure. It is generally situated very near the center of the parasite, but is often also at some distance from the center. It consists of a definite nuclear membrane enclosing a small, deeply stained dot, the karyosome.

The nucleus is small in relation to the total size of the endameba. This statement is agreed with by practically all observers who have studied the organism, but the measurements given by these observers vary considerably. Von Prowazek stated that the nucleus measured from 1.5 to 4.5 microns in diameter, while Smith and Barrett give the measurement as from 2 to 5 microns in diameter. My experience has been that it is very rare for an individual of this species to show a nucleus exceeding 3.5 microns in diameter and that the average diameter of the nucleus is about 3 microns. The nucleus of this species is considerably smaller than the nuclei of Endamoeba coli and Endamoeba histolytica and very noticeably so in the large vegetative forms of the parasite. The smallness of the nucleus of Endamoeba gingivalis is a very valuable differential point when one is comparing it with other species of endamebae.

The shape of the nucleus is generally circular, but it may be oval or elongated. The nuclear membrane stains black and is well defined in properly prepared specimens, but in those in which differentiation with the iron-alum solution has been carried too far, the membrane may be very dim in outline. The thickness of the membrane also varies with the amount of differentiation — a fact that has caused some confusion in the description of the nucleus, some authorities claiming that the nuclear membrane is thick, others that it is very thin and delicate. If properly differentiated the nuclear membrane of Endamoeba gingivalis is intermediate in thickness between that of Endamoeba coli and that of Endamoeba histolytica, being thinner than that of the former and thicker than that of the latter. In well-prepared specimens the nuclear membrane should appear as a distinct, black circle bounding the nucleus.

The nucleus of this species is very poor in chromatin and in the majority of instances none of this substance can be distinguished within the nucleus, with the exception of the small, black mass, situated centrally, which forms the karyosome. Sometimes, however, the nuclear membrane shows a slight thickening at one or more parts of the periphery, and minute grains of chromatin, stained black, may be observed upon the inner surface of the membrane. In very rare instances a few minute grains may be observed lying between the nuclear membrane and the karyosome, but this is very exceptional. In carefully differentiated specimens a very delicate linin net-work may be observed between the nuclear membrane and the karyosome, imbedded in which there may be one or more dots of chromatin stained dark-brown or black. However, in the majority of instances, the nucleus is composed simply of a well-defined nuclear membrane enclosing a space filled with amorphous, grayish-stained material, at the center of which is a small, deeply stained karyosome.

The general appearance of the nucleus of the vegetative endamebae of this species is very like that of the nucleus of Endamoeba histolytica during the histolytica stage of development. The resemblance is so great, in fact, that Smith and Barrett have brought up the point of the possible identity of the two species; but, in my opinion, the nucleus of Endamoeba gingivalis can be distinguished from the histolytica type of nucleus of Endamoeba histolytica if attention be paid to its smaller size, its thicker nuclear membrane, and its larger karyosome. Of course, individual endamebae occur in which the nucleus is anomalous in some respect, and in such instances a differentiation might be impossible; but, in the vast majority of instances, one should have little difficulty in differentiating the nucleus of Endamoeba gingivalis from that of Endamoeba histolytica, in well-stained preparations. It is also a fact that in no example of Endamoeba gingivalis that I have studied, has a nucleus at all like that of the tetragena type of the nucleus of Endamoeba histolytica been observed, nor have I seen it stated by an observer that such a type of nucleus occurs in this species. Certainly, if the two species were identical, the tetragena type of nucleus would sometimes occur.

The karyosome in this species is small and stains a deep brown or black. It generally consists of a small, compact mass, altho it rarely may appear granular and be composed of several deeply stained grains of chromatin. A centriole has not been observed. In size the karyosome is smaller than that of the nucleus of Endamoeba coli and larger than that of Endamoeba histolytica. If specimens are overdifferentiated in the iron-alum solution, the karyosome may appear to be as small as that of the histolytica type of nucleus of the latter organism, and, in fact, it should be remembered that the appearance of the nucleus of any of the endamebae may be made to resemble that of another

species by variations in the time of staining and the amount of differentiation, so that it is not surprising that observers have differed in their interpretations of the morphologic structure of so minute an object as the nucleus and have formed erroneous conclusions regarding the resemblance of the nucleus of one species to that of another. However, it is true that long practice in the staining of these organisms and the examination of a large amount of material render it possible to differentiate the principal species of endamebae that have been described by their nuclear structure, and Endamoeba gingivalis is no exception to this rule.

The cytoplasm of this species, during the vegetative stage, stains a dull-grayish color and is generally granular in appearance. If the endamebae are small, ingested bodies are not so frequently observed, but, as a general rule, numerous vacuoles are present, many of them containing the debris of ingested bodies, or oval, black bodies, often larger than the nucleus of the endameba, which have been interpreted by most observers as the nuclei of ingested leukocytes. In addition, red blood corpuscles are sometimes seen within the cytoplasm, but these occur much less frequently than one would be led to believe from the usual descriptions of this species.

One of the most characteristic morphologic features of the vegetative stage of development of this species is the very large number of parasites which are filled with oval or round bodies, stained black, and surrounded by a well-marked unstained area. These bodies are evidently contained within vacuoles. They vary in size from 1 micron in diameter to as much as 4 or 5 microns. Altho interpreted by most observers as the nuclei of ingested leukocytes, careful observation will demonstrate that they are not all of the same nature, and that while some are undoubtedly of leukocytic origin, the majority are not derived from these cells, but represent some form of yeast or protozoan organism. Whatever their exact nature, they are generally present in the vast majority of the vegetative forms of Endamoeba gingivalis, and, while not absolutely characteristic of this species—for the same bodies may be observed in both Endamoeba coli and Endamoeba histolytica—they do occur so frequently in this species, and in so many of the organisms, that their presence is of some diagnostic value. It may be stated that I have never observed these bodies within the new species of mouth endameba already referred to as Endamoeba confusa—a fact that helps to distinguish the larger forms of this species from Endamoeba gingivalis.

The vacuoles, with which the cytoplasm of Endamoeba gingivalis is so often filled, vary considerably in size in the individual endamebae, but in the smaller vegetative forms they may be all of about the same size and so numerous that the entire cytoplasm appears to be composed of a net-work of dimly stained material enclosing small, unstained, round or oval areas, the vacuoles. In such instances the vacuoles do not appear to contain ingested material, but in most of the organisms one or more larger vacuoles are present which show ingested bodies.

Reproductive vegetative forms are, in my experience, rarely encountered in stained preparations. I have several times observed endamebae containing 2 nuclei or organisms in which the nucleus was evidently undergoing mitosis, but when compared with the large number of endamebae studied the reproductive forms were singularly rare, even when a very careful search was made for them. This is rather surprising, because in favorable cases the vegetative forms of this species are very numerous and one would expect to find many

undergoing division, but tho such infections were followed from day to day it was the exception to find organisms showing any evidence of division and two-nucleated endamebae were very rare in such massive infections.

When 2 nuclei were observed in a single individual of this species, they were of about the usual size. The nuclear membrane in each case was distinct and the karyosome well stained and situated at the center of the nucleus. The nuclear membrane appeared slightly thinner than it is when only one nucleus is present, and I have never observed any chromatin upon the inner side of the membrane, or free chromatin between the membrane and the karyosome.

A primitive form of mitosis occurs in the nucleus of this species, as the karyosome has been observed to separate into 2 parts connected by a delicate thread of chromatic material; rarely a more or less distinct nuclear spindle may be demonstrated. After the division of the karyosome the nucleus becomes elongated and a constriction occurs near the center, which increases until the nucleus is divided into 2 portions of equal or nearly equal size. Various stages of this process may be traced in stained preparations but only with difficulty, owing to the relative infrequency of dividing forms.

I have several times observed individuals of this species which have been interpreted by some authorities as examples of reproduction by budding or gemmation, but it is my belief that this process is a degenerative one in this species, just as it is in Endamoeba histolytica. Organisms are observed in stained preparations which contain granules, rods, and clumps of chromatic material within the cytoplasm, while the nucleus has either disappeared or appears to be breaking up and supplying this material to the cytoplasm. Sometimes the deeply stained chromatic material appears to be collected near the periphery of the parasite and small projections containing some of the same material may be seen apparently being budded from the periphery, but the morphologic details are exactly the same as those observed in the so-called budding forms of Endamoeba histolytica and there is no more reason to believe that this is a reproductive process in Endamoeba gingivalis than that it is in Endamoeba histolytica, where it has been proved to be degenerative in character.

The Pre-Cystic Stage.—The pre-cystic forms of this species, in stained preparations, are characterized by their small size, homogeneous cytoplasm, and the larger size of the nucleus in comparison with the total size of the parasite. These forms have not been described before, evidently having been mistaken for vegetative forms. They do not occur very frequently and are rare even in those infections which present them.

The pre-cystic stained forms seldom measure more than 10 microns in diameter; the cytoplasm is homogeneous in appearance, finely granular in structure, and stains a brownish gray or distinct gray with the method described. No vacuoles are present, in most instances, and the cytoplasm is free from ingested bodies. The nucleus is large in relation to the total size of the parasite, generally measuring over 4 microns in diameter, and frequently as much as 5 or 6 microns. The nuclear membrane is well defined, staining a dark brown or black, and is slightly thicker than it is in the vegetative forms. The karyosome stains black, is situated at the center of the nucleus, and is larger than it usually is in the vegetative forms. The space between the karyosome and the nuclear membrane stains a dim gray and is generally free from chromatin, altho in some instances a few small, black granules may be seen in this space or upon the inner side of the nuclear membrane. At this stage of development there is no sign of a cyst wall. I have never observed any evidence of reproduction in these pre-cystic forms and it is my belief that they are merely individuals that are about to encyst.

The Cystic Stage.—The cystic stage of Endamoeba gingivalis was first mention by Chiavaro,[7] but he does not describe the cysts and does not state whether this is a reproductive process in this species, altho he pictures a cyst containing 2 nuclei. It must be said that his two drawings of the cystic forms of this species are not at all convincing and it is most unfortunate that no description of the morphology of the cysts is given in his paper. The only other authors to mention cysts in this species are Smith and Barrett,[14] who state that they have observed "dauer cysts" but no reproduction cysts, and no description is given in their article of the "dauer cysts."

In my experience the cystic forms of this species are very rarely observed, the conditions in the mouth apparently being most favorable to the development of the vegetative forms. I have also found that, when present, they occur in very small numbers, the vegetative forms, even in such cases, far out-numbering them.

The cysts measure about 10 microns in diameter, but examples are observed which measure as much as 12 microns. The cytoplasm appears homogeneous and finely granular and stains a dark brown or brownish gray with the method described. Vacuoles and ingested bodies are absent, as in the pre-cystic forms; in rare instances I have observed cysts containing a single, large vacuole, but when fully developed the cysts are free from vacuoles. The nucleus is well defined, generally situated at or near the center of the cyst, altho it may be placed at the periphery. It varies in size but generally measures 3 to 4 microns in diameter, most of the cysts showing a nucleus smaller than that observed in the pre-cystic forms. The nuclear membrane stains a deep black and seldom presents any chromatin dots on its inner surface, being perfectly smooth and slightly over a line in thickness. The karyosome is composed of a single, small mass of black-stained chromatin, situated at or near the center of the nucleus, while the space between the karyosome and the nuclear membrane contains no chromatin, appears finely granular, and stains a light gray in color.

The cyst membrane varies in appearance apparently with the age of the cyst. At first it is very difficult to differentiate this membrane from the periphery of the cytoplasm, and at this stage of development it is often impossible to tell whether one is dealing with a pre-cystic or cystic form. The first appearance of a membrane consists in a very delicate line surrounding the periphery of the organism; in older cysts this line appears thicker and in some instances a definite double-contoured membrane may be easily distinguished. In my experience the cyst membrane is generally more delicate and more difficult to distinguish in this species than it is in either Endamoeba coli or Endamoeba histolytica.

Reproductive changes, so far as I have been able to observe, do not occur in the cysts of Endamoeba gingivalis, for I have never observed a cyst with more than one nucleus, nor have I seen any changes in the nucleus that could be interpreted as reproductive in character. So far as the evidence goes, cyst formation in this species is purely protective in character, for no investigator has yet given any proof that multiplication of the parasite occurs within the cyst. From the morphology of the cysts observed in stained preparations it is evident that before encystment the endameba frees itself of all ingested material and that the cytoplasm becomes more dense in structure, as it stains much more intensely than does the cytoplasm of the vegetative forms. These phe-

[14] Jour. Parasitol., 1915, 1, p. 167.

nomena are common to all endamebae before encystment, so that this species does not differ, in this respect, from other species described. It may be that further research will demonstrate that reproduction occurs with the cysts, but at the present time there is no evidence that encystment is anything more than protective in nature.

THE IDENTITY OF ENDAMOEBA GINGIVALIS AND ENDAMOEBA HISTOLYTICA

Because of the statements of Smith and Barrett[15] that they were unable to differentiate Endamoeba gingivalis morphologically from Endamoeba histolytica it is necessary to consider briefly this phase of the subject. They state that microscopically they were unable to differentiate the two species, while both reproduce by binary fission, and both fail to produce reproduction cysts. I am unable to agree with these authors as to the morphologic resemblance of Endamoeba gingivalis to Endamoeba histolytica and with their statement regarding reproduction by gemmation in the two species and the absence of reproductive cysts in Endamoeba histolytica.

Endamoeba gingivalis resembles Endamoeba histolytica morphologically only when the latter is in that portion of its life cycle generally known as the histolytica stage. At this time the nucleus of Endamoeba histolytica does resemble, at first glance, the nucleus of Endamoeba gingivalis, but it is much larger in comparison with the total size of the organism, while the nuclear membrane is more delicate and the karyosome considerably smaller. The size of Endamoeba gingivalis averages much smaller than that of Endamoeba histolytica and its motility is much more restricted and less active. The ectoplasm of Endamoeba gingivalis, in living specimens, is never as refractive and glass-like as that of Endamoeba histolytica and its pseudopodia are much smaller.

The form of reproduction by "gemmation" mentioned by Smith and Barrett, has now been definitely proved to be a degenerative process, having nothing whatever to do with reproduction, and the fact that it occurs in this species, as well as in Endamoeba histolytica, simply demonstrates that both species undergo the same form of degeneration.

As is well known, at one stage of its development Endamoeba histolytica presents a very definite type of nucleus, known as the tetragena type; but this type of nucleus has never been observed in Endamoeba gingivalis, no matter what the clinical character of the infection. If the species were identical, such a type of nucleus would most certainly

[15] Jour. Parasitol., 1915, 1, p. 173.

have been observed; for there is no reason to believe that this stage in the development of the species would not occur in the mouth.

The statement of Smith and Barrett that neither species develops reproductive cysts is erroneous so far as Endamoeba histolytica is concerned, for the well-known four-nucleated cysts of this species are relied upon to distinguish this stage in the development of the organism from the cysts of Endamoeba coli, which contain 8 or more nuclei. The fact that the cysts produced by Endamoeba gingivalis have only one nucleus and are therefore probably not reproductive in character, alone serves to distinguish this species from Endamoeba histolytica.

If we add to these differences in morphology and life-cycle the fact that neither rectal injection nor feeding of material containing Endamoeba gingivalis gives rise to diarrhea or dysentery in animals, in which such symptoms are readily produced by Endamoeba histolytica, I believe that the evidence is conclusive that the two species are not identical.

RELATION OF ENDAMOEBA GINGIVALIS TO DISEASE

Altho the assertion that this endameba is the cause of certain diseases of the teeth and gums has been made by numerous authorities, some of whom have even endeavored to trace a relation between Endamoeba gingivalis and certain systemic conditions, it may be stated that at the present time the question is far from settled tho there is a constantly growing opinion among those who have had the largest experience with pyorrhea alveolaris and other diseases of the mouth, that Endamoeba gingivalis has little, if anything, to do with the conditions present, being merely a harmless commensal, as is Endamoeba coli in the intestine.

Despite the previous paper by Lewald,[5] published in 1907, and apparently unknown to Barrett, in which he demonstrated that the species of endameba under discussion could be found, upon careful search, in the mouths of most healthy persons, 71 positive results having been obtained by him in 100 such individuals, Barrett,[6] in conjunction with Smith, published a paper in 1914 in which they asserted that this parasite was probably the cause of pyorrhea alveolaris, basing their conclusions upon the presence of the organism in the lesions of the disease and on the improvement of the condition after the administration of emetin, a well-known amebacide. While the paper of Barrett was conservative, the same cannot be said of the paper by Bass and

Johns,[9] published in 1915, in which these authors state, unconditionally, that pyorrhea is due to endamebae, altho they give no scientific evidence for the statement beyond the finding of the endamebae in the lesions of the disease and the improvement of the condition after the administration of emetin. While the conclusions drawn in these two papers cannot be said to have been proved, they have served to incite research in this direction, being responsible for most of the work by both dentists and physicians upon this organism during the past two years.

Shortly after the publication of Barrett's original paper, Angelo Chiavaro,[7] working in Grassi's laboratory in Rome, published the results of his researches upon Endamoeba gingivalis and its relation to pyorrhea. He found the endameba present not only in the pus of cases of pyorrhea, but also in the cavities of carious teeth and in the materia alba and other deposits upon sound teeth. In his conclusions he states that while this species of endameba is found in the pus of most cases of pyorrhea alveolaris it is also generally present in the materia alba of the sound teeth if they are not kept in hygienic condition, and in carious cavities if the reaction is acid. He states, in conclusion: "The endamoeba has not a pathogenic action; on the contrary, as it feeds on bacteria, it is most probably an aid to the autodisinfection of the mouth."

That this species of endameba is found in a very large percentage of healthy mouths, as first shown by Lewald, is demonstrated by the researches of Anna Williams,[16] who found it in 30% of children with healthy gums. She also found this parasite in 50% of children with healthy gums and carious teeth; in 84% of children showing tartar around the teeth and receding gums; and in 94% of children with spongy and bleeding gums. She makes the following statement:

> We can say nothing definite yet as to the significance of the amebas in these mouths. Finding them so often in apparently healthy mouths, and in such young children, does not agree with the statements of Bass and Johns, and Barrett, that they are not found in healthy mouths.
>
> I have repeatedly found *Endamoeba gingivalis* in the materia alba around perfectly sound teeth and in perfectly healthy mouths, and have failed to find the parasite in some typical cases of pyorrhea, and while this parasite undoubtedly occurs more frequently, or, at least, is more easily demonstrated when the gums are diseased, its mere presence is certainly no proof of its etiological relationship to pyorrhea alveolaris or other diseases of the mouth.

[16] N. Y. Path. Soc., March, 1915. Cited by Merritt, New York Med. Jour., 1915, 102, p. 281.

Since the publication of the original paper of Barrett, the treatment of pyorrhea with emetin, based upon the supposed causation of the disease by endamebae, has been vigorously pursued by the dental profession throughout the country, and it must be admitted that the reports regarding its efficiency are most conflicting and the more recent articles most disappointing regarding the value of the drug as a specific for this disease. Such dental authorities as Henrici and Hartzell,[10] Sarrazin,[11] and Merritt[17] believe that the emetin treatment of pyorrhea is not curative and that there is no evidence sufficient to prove that the disease is due to endamebae. Hartzell states that, in his hands, emetin has failed even to benefit the condition, while Sarrazin saw no decided improvement in the patients under his observation. Merritt is inclined to believe that what benefit does follow the use of emetin in pyorrhea is due to the fact that it is a hemostatic and concludes: "Whatever its action may be, there is at present no trustworthy evidence that it will cure pyorrhea."

Through the kindness of Dental Surgeon Minot E. Scott, U. S. Army, I have had the opportunity of following several patients suffering from pyorrhea alveolaris who were being treated with emetin, and I have found that while the endamebae generally decrease in number during its administration, careful search will show motile and apparently healthy endamebae in the lesions throughout the period of administration. In one instance there was no appreciable decrease in their number altho the drug was exhibited in the most approved manner for several weeks.

It should also be remembered that emetin is not without action upon other organisms, as has been shown by Vedder,[18] so that all the improvement following its use in pyorrhea cannot logically be credited to its amebacidal action, especially when the endamebae do not disappear tho the symptoms improve, as has happened in some instances. In all these cases I have found that spirochetes are present in immense number and it is just as reasonable to believe that these cause pyorrhea and that the emetin has some action on them (as they certainly lessen in number under treatment) as it is to claim that endamebae are the cause of the disease because of their presence before, and decrease after, the administration of this drug.

Price and LaRue,[12] in a summary of the present status of the emetin treatment of pyorrhea, prepared for the Scientific Foundation

[17] New York Med. Jour., 1915, 102, p. 279.
[18] Bull. Manila Med. Soc., 1911, 3, p. 48.

and Research Commission of the National Dental Association, after considering the arguments for and against the theory of the causal relationship of Endamoeba gingivalis to pyorrhea alveolaris, state that they have observed many cases of severe typical pyorrhea in which this endameba could not be demonstrated, and that in many cases of the disease emetin gives very poor or no results. In their opinion the proof is yet insufficient that these organisms have anything whatever to do with the production of the disease.

At the present time the only proof we possess of the etiologic relationship of Endamoeba gingivalis to pyorrhea alveolaris consists in its almost constant presence in the lesions of the disease and in the fact that emetin, properly administered, greatly benefits a large majority of the patients suffering from the disease. There is absolutely no experimental proof of the etiologic relationship of this parasite to pyorrhea, while against this relationship we have the following facts: The occurrence of the parasite in a large percentage of healthy mouths and in the material that can be scraped from healthy teeth and gums; the occurrence and persistence of the parasite in patients treated with emetin, even when marked improvement in the clinical symptoms has occurred; the absence of the parasite in some typical cases of pyorrhea; the lack of improvement with emetin shown in numerous instances of the disease, altho the endamebae may disappear; and the fact that emetin acts upon other organisms as well as upon endamebae and the possibility that the improvement that often follows its administration may be due to such action or to a favorable action upon the tissue cells.

In conclusion, it may be stated that if one considers carefully the data that have accumulated upon this subject there is but one deduction which can be drawn, and that is that it is more than doubtful that Endamoeba gingivalis is the cause of pyorrhea alveolaris, and that it is yet too early to make positive statements regarding the relationship of this parasite to disease.

EXPLANATION OF PLATE 6

Fig. 1. Young form of Endamoeba gingivalis. Note size of nucleus, and homogeneous cytoplasm, which contains some bacteria.

Figs. 2, 3, and 4. Vegetative forms of Endamoeba gingivalis, showing the ingested bodies so frequently observed. Note the size of the nucleus in relation to the total size of the endameba.

Fig. 5. Dividing form of Endamoeba gingivalis, containing ingested bodies and bacteria. Note minute size of the two nuclei.

Fig. 6. Dividing form of Endamoeba gingivalis with unusually large nuclei. In this organism the nuclei closely resemble the nucleus of Endamoeba coli.

Figs. 7 and 8. Vegetative forms of Endamoeba gingivalis. In Fig. 7 the nucleus is very typical. In Fig. 8 the nuclear membrane is thickened at two portions of the periphery.

Figs. 9 and 10. Pre-cystic forms of Endamoeba gingivalis. Note larger size of the nucleus in these pre-cystic forms and the homogeneous cytoplasm.

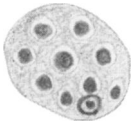
Fig. 11. Cystic form of Endamoeba gingivalis showing single outline to the cystic membrane. Note smaller size of the nucleus and homogeneous, deeply stained cytoplasm.

Fig. 12. Cystic form of Endamoeba gingivalis, showing double-contoured membrane.

Plate 6

1

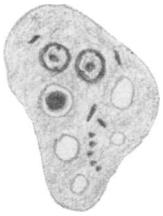
2



3

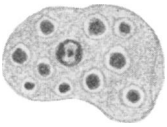
4

5



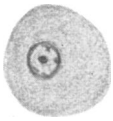
6

7



8

9



10

11

12